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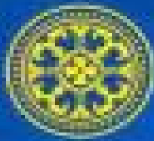

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MAINTAINING WORLD PROSPERITY THROUGH BIOSCIENCES, BIOTECHNOLOGY AND REVEGETATION

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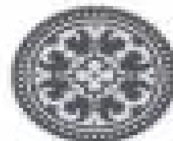

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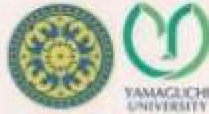

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Head of Organizing Committee

Pattern of Influenza Virus in Denpasar During Influenza-like Illness Survey in 2009-2011

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Abstract

Background

Influenza is considered one of the most important infectious diseases. Complicating the global burden is emergence of new strain of virus during H1N1 outbreak, we now experiencing of the 1st pandemic in this century. Although Influenza illness is usually mild, but changes in their genetic material will create more pathogenic virus than its origin. The aim of this study is to know the seasonal pattern of Influenza in Denpasar during actively Influenza-like Illness surveys in 2009-2011. Subject of this study was collected in two group, for the surveillance cases, sample was collected from Denpasar Selatan I Primary Health Care Center. While for the outbreak, sample was collected from Sanglah Hospital Denpasar. Sample was collected from the nasal and throat specimens. The specimens were transported using Hank medium. In the laboratory, specimens were extracted using Invitrogen Extraction Kit. PCR used was Superscript III Platinum One-Step qRT/PCR System Real-time PCR. For the surveillance specimen, we determined Influenza A and Influenza B type and H1 seasonal, H3 and H5 subtype. While for the outbreak we only determine the H5 subtype for Avian Influenza Outbreak and H1 novel for Swine Flu Outbreak.

During August 2009 until June 2011 period we collected 832 specimens. In a period of August 2009-June 2010, a total 381 specimen was collected, among them 25 (7%) was positive for Flu A, 8 (2%) positive for Flu B and 13(3%) positive for H3 while the 335 (88%) was included in non subtype. On the other hand during July 2010 until June 2011, a total of 451 specimens were collected, 17(3,8%) was positive flu A, 25(5,5%) Positive flu B, 2(0,4%) positive H3 and 7 (1,6%) positive for H1. During 2009-2010 Flu A was detected fluctuated, while flu B detected only in June and H3 detected highest in April 2010. During 2010-2011 Flu A was detected in January, 2011, Flu B was detected highest in September

2010 and Swine flu (H1) detected highest in March,2011. H1 and H5 were not detected in this survey period.

We can conclude that during this 2 years period of survey Flu B was found increased during 2010-2011, and Swine flu (H1) only found during outbreak in 2010-2011.

Key words: ILI, Flu A, Flu B, Denpasar.

INTRODUCTION

The emergence of pandemic influenza, whether bird flu (H5N1) or swine flu (H1N1) in Indonesia in 2006 and 2009, making the government and health department are increasingly recognizing that influenza is not just a common disease but also can be very deadly.

Influenza is a disease caused by a virus belonging to the family Orthomyxoviridae (Murphy,*at all*.,2001). Based on differences in the matrix protein (M protein), influenza viruses can be divided into several types of influenza type A, B and C. Influenza type A is further subdivided into several subtypes based on antigenic differences in the external glycoprotein that called Haemagglutinin (HA) and Neuraminidase (NA). Until now there are at least 15 types of HA and 9 different NA types have known. However, only a few subtypes of influenza A, that cause disease in humans such as H1-, H3- and H5- subtype. The N subtypes that usually fatal such as N1- and N21. Indonesia has an experience of Influenza outbreak in 2006 and 2009 that cause by H5N1 and H1N1 subtype.

Clinical manifestations of influenza that is often similar to respiratory diseases caused by other pathogens, causing the disease was difficult to diagnose when only based on clinical symptoms. To overcome this difficulty, several methods have been developed, such as viral genetic isolation, antigenic examination, and molecular biology. Each of these diagnostic methods has its own drawbacks and advantages as can be seen from Table 1.

Tabel.1. Comparison of Influenza virus diagnostic method.

Method	Target	Specimen	Sensitivity	Specificity	Time
RT-PCR	RNA	Swab, Tissue	+++++	+++++	< 3 day
Virus Isolation	Virus	Swab, Tissue	+++++	+++++	2-5 day
Antigen Examination	Antigen	Swab, Tissue	++	++	1 day

Rapid and accurate method of diagnosis would help the clinician to perform the right and appropriate therapy and also help the health officer to monitoring and surveillance of ILI cases in community. This is supported by the program that is currently developed by the Agency for Health Research and Development (Balitbangkes) Ministry of Health Republic of Indonesia by pointing to several laboratories in the region as Regional Influenza Laboratory. Since 2007, Section of Microbiology Faculty of Medicine, Udayana University was appointed as the Regional Influenza Laboratory for Bali and Nusa Tenggara. By forming the Regional Laboratory, the government expects to have sufficient data for the spread of influenza cases in Indonesia.

The purpose of this study was to determine the frequency of influenza viruses from specimens that was collected from the patient with ILI symptom (Fever > 100F, with cough and/or Shore throat) who comes to the Denpasar Selatan 1 Primary Health Centre and RSUP Sanglah Denpasar.

METHODS

This study is a descriptive study. The study was conducted at the Molecular Biology Laboratory Faculty of Medicine, Udayana University from August, 2009 until June 2011. The specimens were collected from Denpasar Selatan 1 Primary Health Centre and Sanglah Hospital. The samples were divided into 2 groups: samples derived from surveilance cases and samples from outbreak cases. For the case of surveillance, samples derived from patients who has ILI

symptoms and went to Denpasar Selatan 1 Primary Health Centre and for the case of an outbreak, a sample derived from Sanglah Hospital Denpasar. The specimen was taken from the nose and throat swabs, then placed into transport medium of Hank's media and further processed in the laboratory.

RNA ISOLATION

For surveillance specimens, specimens were extracted using the QIAmp ® Viral RNA Mini Kit (Qiagen) using outbreak specimens while the Pure Link ™ Viral RNA / DNA Mini Kit (Invitrogen).

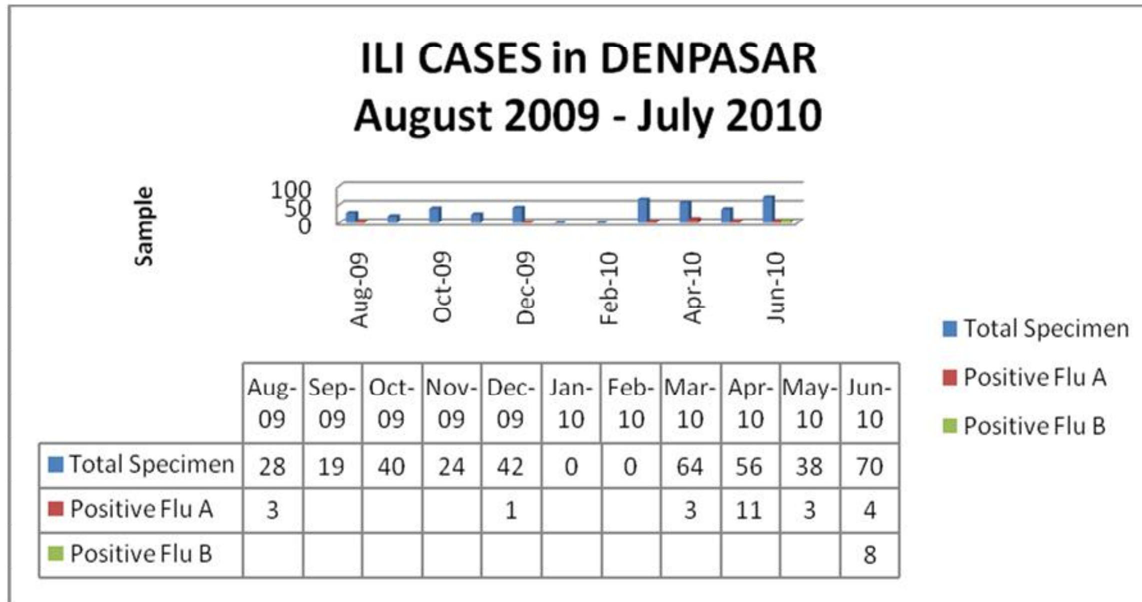
Real-time PCR

Primers used for PCR process was provide by Balitbangkes according to CDC Atalanta protocol. For surveillance specimens Real-time PCR was used AgPath-ID ™ One-Step RT-PCR Kit (Ambion), while for outbreak specimens used Superscipt III Platinum One-Step qRT / PCR System Real-time PCR Kit (Invitrogen). Differences reagents used for each of these specimens affect the cycle time used in the PCR process. In surveillance specimens, PCR tube already containing 25µL PCR mix and mold incorporated into real-time PCR machine IQ-5 (Biorad) with a cycle of reverse transcriptase (RT) at 50 ° C for 30 minutes, pre-denaturation at 95 ° C for 10 seconds and proceed with the process of denaturation and annealing respectively at 95 ° C for 15 sec and 55 ° C for 30 seconds as many as 45 cycles. The difference lies in the process of pre-cycle denaturation where for the outbreak specimen took 2 minutes within the same temperature.

For examination of Surveillance specimens, we determined the type of Influenza A and Influenza B, followed by examination of seasonal subtype H1, H3 and H5. As for the specimens' outbreak, we just check for the H5 subtype of bird flu outbreaks and the H1 subtypes for novel swine flu outbreak.

RESULTS AND DISCUSSION

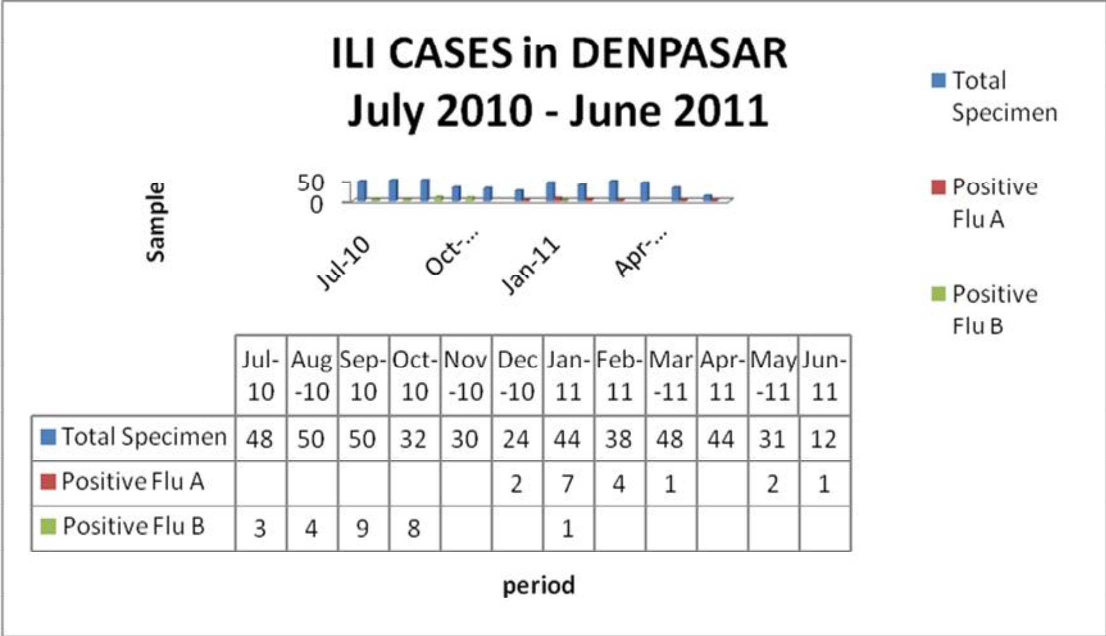
During the surveillance period (August 2009-june 2011), there are a total of 851 specimens was collected. Three hundred eighty one specimens were collected during August 2009- June 2010, while 451 specimens collected during July 2010 – June 2011. Among them total of 40% was female and 60% male, which age range was from 1 month – 49 years old.



	Aug-09	Sep-09	Oct-09	Nov-09	Dec-09	Jan-10	Feb-10	Mar-10	Apr-10	May-10	Jun-10
Total Specimen	28	19	40	24	42	0	0	64	56	38	70
Positive Flu A	3				1			3	11	3	4
Positive Flu B											8

Graph 1. The number of ILI Surveillance Specimen August 2009-July 2010

Among 381 specimen was collected, during surveillance of ILI cases in August 2009-July 2010, 25 (6,6%) positive Flu A and 8 (2,1%) positive for flu B. the high number of specimen was collected in first four month of 2010. Increasing the number of cases was due to in this month was rainy season. In January and February 2010, there is no samples was collected, this is not due to no cases of ILI during this 2 month, but because of technically problem.



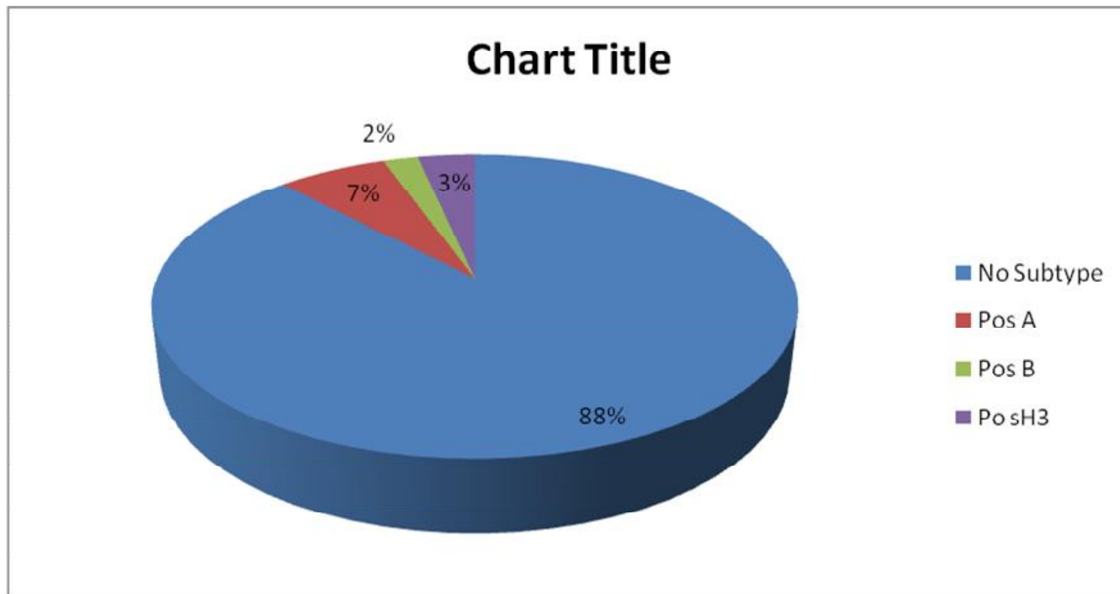
	Jul-10	Aug-10	Sep-10	Oct-10	Nov-10	Dec-10	Jan-11	Feb-11	Mar-11	Apr-11	May-11	Jun-11
Total Specimen	48	50	50	32	30	24	44	38	48	44	31	12
Positive Flu A						2	7	4	1		2	1
Positive Flu B	3	4	9	8			1					

Graph.2. The Number of ILI Surveillance Specimen July 2010 – June 2011

Seventeen (3,8%) of 451 specimen collected during July 2010 – June 2011 was positive Flu A and as much as 25 (5,5%) specimen was positive Flu B. One case of Flu B was isolated in 2011. Data of flu B in 2011 isn't a representative of Flu B in a year, because fluctuation of cases is always changes. The interesting data is a pattern when the Flu A isolated there is no Flu B was found and vice versa. This situation indicating that in a year one of them will dominant.

During this two year surveillance, specimen positive for Flu A was found all the year, most often isolated in March to June each year. On the other hand specimen which is positive for flu B, was found in June – October 2010. The association between season and pattern of Influenza A and B hasn't understood yet, because we don't analysis that association. This result is in line with data obtained in Jakarta and surrounding areas. Djoko *et al* (2009), using an different antisera and hemaglutination inhibition found that influenza A was dominantly, and small number of influenza B. From research conducted by Agrawal *et al* (2009), data from surveillance of children in India acquired 11.09% of influenza A and influenza B 5.41% of total 1091 samples.

Hadzhiolova et al (2006) in Bulgaria in 2004/2005, obtained 77 clinical samples consisting of 13% of positive influenza A subtype H3N2 and 24.6% of positive influenza subtype H1N1. But to note, that in this research in Bulgaria, Hadzhiolova still using the conventional PCR method by using gel electrophoresis in the examination.



Graph 3. Influenza Surveillance Data in Bali the period August 2009-June 2011

From 851 specimen that we have had analysis, among them 7% positive for Flu A, 3% positive for H3, and 2 % positive for Flu B and 88% was no-subtype. The high number for no-genotype can be due to the sensitivity of the method that can use to detect, the reagent that use was in appropriate. Upper respiratory infection can be caused by bacteria, virus or fungi. Most of them is virus. In this research we just identified for Influenza virus, in negative specimen there is might be virus other than influenza virus (Rhinovirus, parainfluenza virus, and respiratory sinctial virus) or mutated influenza virus.

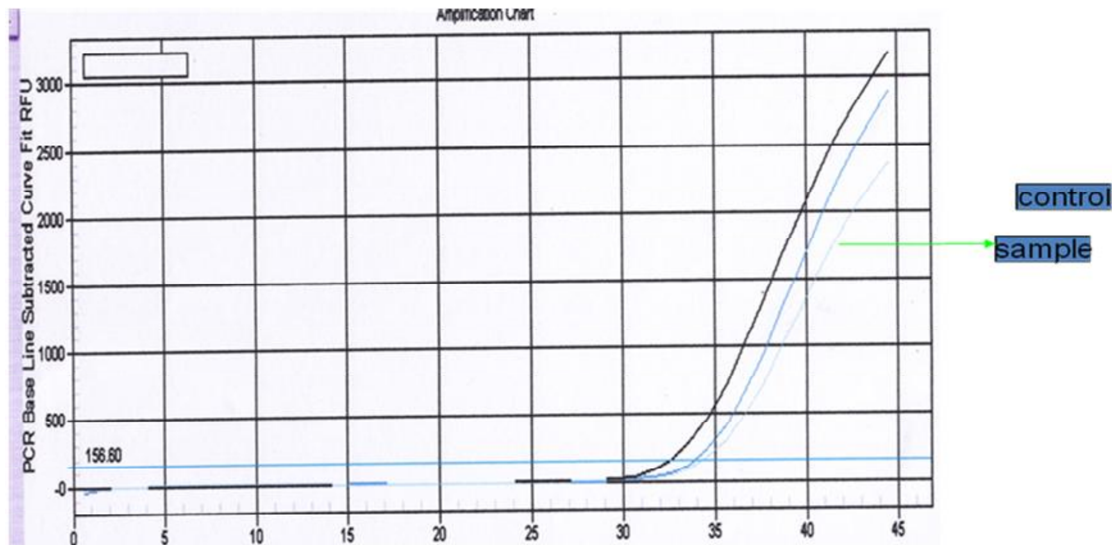


Figure.1. Real Time PCR result

Over the past two decades, virus isolation and serology have been the mainstay of the clinical laboratory for diagnosing respiratory virus infections. A variety of serological test including the hemagglutination inhibition (HAI) test, complement fixation, and enzyme immunoassay (EIA) were used for testing paired acute- and convalescent-phase sera for diagnosing viral infections. Currently,viral culture usually in combination with immunofluorescence (IF) is the “gold standard” for laboratory diagnosis. However, it is not a rapid diagnostic test, and therefore, its clinical value is limited. Now days Nucleic Acid based diagnostic was use to detected respiratory viral infection (Mahony et al.,2008). Real-Time PCR methode in combination with cell culture increase the sensitivity to detected respiratory viral infection (Strorch.,2003)

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